Effect of vitamin D treatment in hypoparathyroid patients: a study on calcium, phosphate and magnesium homeostasis

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Abstract

Aim: This study was undertaken to examine the effects of long-term vitamin D treatment on calcium, phosphate and magnesium homeostasis at organ level in hypoparathyroid patients.

Methods: Fifteen vitamin D-treated hypoparathyroid patients were studied, eight of the patients in a combined $^{47}$Ca kinetic and calcium, phosphate and magnesium balance study. Results were compared with a matched control group of 12 normal individuals.

Results: All the patients had normal serum levels of calcium, phosphate and magnesium. Absolute intestinal calcium absorption was increased ($P < 0.0001$). Urinary calcium excretion was normal, but active tubular calcium reabsorption ($T_{Ca}/$glomerular filtration rate) was low ($P < 0.001$). Bone resorption rates and bone mineralization rates were very low ($P < 0.001$ and $P < 0.05$). Twenty-four-hour urinary hydroxyproline excretion and serum cross-linked carboxyterminal telopeptide of type I were in the upper normal range. Serum alkaline phosphatase was normal, but serum carboxyterminal propeptide of human type I procollagen and serum osteocalcin were significantly reduced ($P < 0.05$). Calcium balance was positive and significantly different from controls ($P < 0.001$). All parameters from phosphate homeostasis were normal. Intestinal magnesium absorption was low though not significantly different from normal ($P = 0.06$). Urinary excretion of magnesium was not significantly higher than normal, but renal magnesium reabsorption was reduced ($P < 0.001$). Magnesium balance was low, though the difference was not significant ($P < 0.06$).

Conclusion: Long-term vitamin D treatment in hypoparathyroid patients resulted in a positive calcium balance. Bone turnover was very low. Results of bone markers and resorption rate were conflicting. Vitamin D treatment apparently normalized the abnormalities previously found in phosphate homeostasis of hypoparathyroid patients. Magnesium homeostasis was disturbed, with a more negative balance compared with normal subjects, implying a state of magnesium deficiency in these patients.

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Introduction

Hypoparathyroidism (HP) can be defined as ‘a condition with lack of parathyroid hormone effect’ (1, 2). Regardless of the cause of HP, the generally accepted treatment of these patients is to normalize the low serum calcium levels by daily vitamin D supplementation, either as calciferol or 1α-hydroxylated vitamin D. It is, however, not known whether the vitamin D supplementation is able to normalize mineral homeostasis or bone metabolism, or whether vitamin D or its metabolites are capable of substituting the effects of parathyroid hormone (PTH), as the two hormones have different target cells, receptor binding and post-receptor effects (2–9). In order to examine these questions, we performed a combined $^{47}$Ca kinetic and calcium, phosphate and magnesium balance study in a group of hypoparathyroid patients in long-term treatment with vitamin D supplementation. The aim of the study was to describe calcium, phosphate and magnesium homeostasis and bone metabolism at organ level in hypoparathyroid patients treated long-term with vitamin D.

Subjects and methods

Study subjects

The patient group included 15 patients with HP. 11 women and 4 men, mean age = 60 years (range 36–77 years). Four of the patients suffered from idiopathic HP. 11 patients had postsurgical HP with a mean (and median) time since surgery of 20 years (range 2–53...
years). The diagnosis was based on low serum PTH levels on several occasions, relief from muscular spasms by treatment with calcium and vitamin D and inability to maintain normal serum calcium levels, and a rapid return of symptoms when treatment was withdrawn. All patients suffering from postsurgical HP had spontaneously normal thyroid function measured by serum thyrotrphin, serum total thyroxine and serum total tri-iodothyronine. In order to keep serum levels of calcium within the normal range and patients free of muscular symptoms, all patients were treated with daily supplementation of calcium and vitamin D. Eleven patients received 1α-hydroxylated vitamin D tablets (mean 1.5 μg/day, range 0.5–3.0), and 4 patients received calciferol oil (32 000–88 000 IU/day = 800–2200 μg/day). All patients received calcium supplementation, mean 1500 mg elemental calcium/day. None of the patients suffered from other diseases with influence on mineral or bone metabolism or received medication with such effects. None of the women had ever received oestrogen supplementation. All patients had normal liver and renal function. A subgroup of 8 patients, 6 women and 2 men aged 36–70 years (mean = 57 years), were examined in a combined 47Ca kinetic and mineral balance study. Five of these patients suffered from postsurgical HP and 3 from idiopathic HP. Six patients received 1α-hydroxylated vitamin D and 2 received calciferol.

The control group consisted of 12 sex- and age-matched normal healthy volunteers receiving no medication with known influence on mineral or bone metabolism. All persons in the control group were examined in the combined 47Ca kinetic and mineral balance study, but no urine on gelatine-free diet for determination of urinary excretion of hydroxyproline (OHP) was obtained.

**Combined 47Ca kinetic and calcium, phosphate and magnesium balance study**

The combined 47Ca kinetic and mineral balance study was performed using a combination of the modified expanding calcium pool model and a conventional balance study (10, 11). During a 7-day equilibration period followed by a 7-day study period the patients were on a calcium and energy fixed diet. In order to avoid regulatory adaptations due to changes in dietary calcium, phosphate and magnesium the diet was as close to the patients’ daily diet as possible. The diet was based on an individual 4-day dietary record followed by an interview with a trained dietician. During the study period food serving as well as calcium supplementation was doubled—one for the patient and another for analysis. Urine and faeces were collected daily. The compliance in urine collection was monitored by daily measurement of urinary creatinine excretion. The faecal collection was controlled by giving non-absorbable 51Cr in tablets three times daily. In order to define the faecal collection period, carmine red was given orally at the start and end of the collection period. Calcium, phosphate and magnesium content in daily samples from serum, diet, urine and faeces were estimated—calcium and magnesium by absorption spectrophotometry. The content in diet and faeces was estimated in dry ashed samples redissolved in hydrochloric acid, and the urine was acidified. The fasting values of calcium, phosphate, magnesium and creatinine were determined in blood and urine on day 3 of the study.

The kinetic study was started by giving 47CaCl2, 0.74 MBq (20 μCi) intravenously. Blood samples were obtained after 5, 10, 15, 20, 30, 45, 60, 120, 180 and 360 min and after 1, 2, 3, 4, 5, 6 and 7 days. 47Ca activity was determined in daily blood, urine and faecal samples, analysed in a scintillation well counter. Twice daily the whole body 47Ca retention was determined in duplicate on a whole body counter. 47Ca kinetic data were calculated according to the previously described modified model of the expanding calcium pool (10, 11). Endogenous faecal calcium (e), i.e. the net calcium lost via digestive juices, was calculated from the difference between serum and faecal 47Ca-activity curves. Bone mineralization rate (m) was determined by computer from whole body retention curve, according to the model (10, 11). Dermal calcium loss (d) was estimated as the difference between the 47Ca retention estimated by the whole body counting and 47Ca excretion calculated from urinary and faecal excretion. Balance (B) was calculated as dietary mineral content (D) minus the sum of the mineral content in faeces (F), urine (U) and sweat: B = D − (F + U + d). This external balance is in a steady state similar to the internal balance, defined as B = m − r, and from this bone resorption rate (r) can be calculated. Net intestinal absorption of calcium, phosphate and magnesium (b) was calculated as D−F, and expressed as the fraction of dietary intake called beta (β). Intestinal calcium absorption corrected for endogenous calcium loss, the true calcium absorption (a), was calculated as D = (F − e), and calculated as the fraction of dietary calcium called alpha (α).

**Biochemical markers**

Serum parathyroid hormone (PTH(1–84)) was measured by an immunoradiometric assay (Allegro Intact PTH, IRMA from Nichol’s Institute, San Juan, Capistrano, CA, USA). Intra- and interassay coefficients of variation were 2–6% and 5–8% respectively.

Serum 1,25-dihydroxyvitamin D was separated from plasma by HPLC after extraction with acetonitrile and then quantified by competitive protein binding assays (Amersham TRK 870). Variations in extraction were corrected by recovery of added tracer. The detection limit was 10 pmol/l.
Biochemical markers of bone formation. Serum alkaline phosphatase (AP) was measured spectrophotometrically using p-nitrophenyl-phosphate as the substrate according to recommendations of the Scandinavian Committee on Enzymes (12). Intra- and interassay coefficients of variation were 1.8% and 3.0% respectively.

Serum osteocalcin was determined by a radioimmunoassay using rabbit antiserum against bovine bone-Gla-protein, with intra- and interassay coefficients of variation of 5% and 10% respectively (13). All samples were obtained in the morning (14).

Serum carboxyterminal propeptide of human type I procollagen (PICP) was measured by a radioimmunoassay from Farmos Diagnostica (Oulunsalo, Finland). The intra- and interassay coefficients of variation were 3% and 5% respectively and the detection limit was 1.2 μg/l (15).

Biochemical markers of bone resorption. Serum cross-linked carboxyterminal telopeptide of type I (ICTP) was analysed by an equilibrium radioimmunoassay. The intra- and interassay coefficients of variation were 5% and 6% respectively (16).

Twenty-four-hour urine OHP excretion was estimated in a 24-h urine sample collected on a gelatine-free diet. The concentration was measured spectrophotometrically with p-dimethylaminobenzaldehyde substrate according to the manufacturer’s instructions (Organon Tecnica, B V Boxtel, Holland). The intra- and interassay coefficients of variation were 10% and 12% respectively.

Renal handling of calcium, phosphate and magnesium

Total renal tubular reabsorption (T) of a mineral is the sum of actively (Tₐ) and passively reabsorbed mineral from urine: T = filtered mineral − excreted mineral = f × S − U × V, where f = ultrafiltrable fraction in serum, S = serum value, U = urinary concentration and V = urinary volume.

Active renal calcium reabsorption (TₐCa) can, when corrected for differences in glomerular filtration rate (GFR) (17–19), be calculated as: TₐCa/GFR (mmol/l GFR) = (f × SₐCa− UₐCa × V/GFR) / (1 − 0.08 logₑ (f × SₐCa / UₐCa × V/GFR)) = 0.56 × SₐCa− (UₐCa × Pₐcrea/Uₐcrea) / 1 − 0.08 logₑ (0.56 × SₐCa / (UₐCa × Pₐcrea/Uₐcrea)).

The active phosphate reabsorption (TₐP/GFR) was calculated from fasting serum phosphate, urine clearance of phosphate and creatinine, corrected according to Bijvoet’s formula (20).

Magnesium is actively and passively reabsorbed in both proximal and distal tubules (21). A TₐMg (maximum value for tubular reabsorption rate of magnesium) has been postulated (17, 19, 22, 23), but far above physiological concentrations of magnesium. It is assumed that magnesium in the area of human physiological concentrations is reabsorbed at a constant rate, and can be calculated from fasting values of serum magnesium, urine magnesium and creatinine clearance (17, 22, 23): T-Mg/GFR = f × SₐMg− (UₐMg × Sₐcrea) / Uₐcrea.

It has been postulated that at very high serum values the T-Mg/GFR is reduced due to tubular secretion (23), but that is not taken into account in this study.

Statistics

Differences between group means were compared by unpaired two-tailed t-test. The level of significance was chosen at P = 0.05.

Ethics

The study was performed according to the Helsinki II Declaration and approved by the local ethical committee.

Results

In all 15 patients serum PTH(1-84) was below the normal range and significantly lower than levels in the control group (P < 0.0001) (Table 1). Levels of serum 1,25-dihydroxyvitamin D were high and significantly increased compared with the control group (P < 0.05) (Table 1). Serum levels of calcium, phosphate and magnesium were normal in the vitamin D-treated HP patients (Table 1).

| Table 1 Serum values (mean and 95% confidence limits (c.l.)) of PTH(1-84), 1,25-dihydroxyvitamin D (1,25-vitD), calcium phosphate, and magnesium in 15 vitamin D-treated hypoparathyroid patients compared with 12 normal sex- and age-matched controls. (Normal laboratory reference ranges are given in brackets.) |
|----------------|------------------|------------------|------------------|------------------|
|                | Hypoparathyroid  | Normal           |                  |
|                | Mean         | 95% c.l.         | Mean            | 95% c.l.         | P value         |
| S-PTH(1-84) (15-60 pg/ml) | 8.1          | 3.6−12.7         | 45.1            | 37.1−54.2       | <0.001          |
| S-1,25-vitD (24−108 pmol/l) | 206          | 84−327           | 80              | 64−95           | <0.05           |
| S-Calcium (2-20−2-52 mmol/l) | 2.25         | 2.13−2.36        | 2.36            | 2.30−2.42       | NS              |
| S-Phosphate (0.8−1.5 mmol/l) | 1.25         | 1.08−1.42        | 1.24            | 1.15−1.33       | NS              |
| S-Magnesium (0.75−1.25 mmol/l) | 0.82         | 0.76−0.88        | 0.84            | 0.82−0.87       | NS              |

S−, serum value.
NS, not significant.
**Table 2** Results (mean and 95% c.i.) from calcium balance and $^{47}$ calcium kinetic studies in 8 vitamin D-treated hypoparathyroid patients, compared with values obtained from a control group of 12 normal age- and sex-matched persons.

<table>
<thead>
<tr>
<th>Hypoparathyroid</th>
<th>Mean</th>
<th>95% c.i.</th>
<th>Normal</th>
<th>Mean</th>
<th>95% c.i.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Ca (D) (mmol/day)</td>
<td>47.4</td>
<td>35.9-58.9</td>
<td>24.8</td>
<td>21.4-28.2</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Faecal Ca (F) (mmol/day)</td>
<td>37.7</td>
<td>26.6-48.8</td>
<td>21.9</td>
<td>18.3-25.4</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Endogenous faecal Ca (e) (mmol/day)</td>
<td>3.8</td>
<td>3.0-4.7</td>
<td>3.1</td>
<td>2.6-3.5</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Intestinal Ca absorp. (a) (mmol/day)</td>
<td>13.1</td>
<td>11.3-15.0</td>
<td>6.3</td>
<td>4.5-8.1</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Intestinal Ca absorp. (a) (fraction)</td>
<td>0.29</td>
<td>0.23-0.35</td>
<td>0.25</td>
<td>0.18-0.32</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Intestinal Ca absorp. (b) (mmol/day)</td>
<td>9.3</td>
<td>7.7-10.9</td>
<td>3.3</td>
<td>1.5-5.0</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Intestinal Ca absorp. (b) (fraction)</td>
<td>0.21</td>
<td>0.16-0.26</td>
<td>0.13</td>
<td>0.05-0.20</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>24-h urine Ca (U) (mmol/day)</td>
<td>7.2</td>
<td>4.4-10.0</td>
<td>5.0</td>
<td>3.9-6.1</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Renal filtered Ca (mmol/day)</td>
<td>149.0</td>
<td>130.3-167.8</td>
<td>158.0</td>
<td>132.1-183.8</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Renal reabsorbed Ca (mmol/day)</td>
<td>141.9</td>
<td>108.7-175.0</td>
<td>153.0</td>
<td>127.3-178.8</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>T(_\text{Ca})/GFR (mmol/l)</td>
<td>1.19</td>
<td>1.14-1.26</td>
<td>1.77</td>
<td>1.70-1.84</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Bone resorp. rate (r) (mmol/day)</td>
<td>1.0</td>
<td>(-2.0)-4.0</td>
<td>7.9</td>
<td>6.7-9.0</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Bone mineral. rate (m) (mmol/day)</td>
<td>2.6</td>
<td>1.6-3.6</td>
<td>4.5</td>
<td>3.1-5.9</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Calcium balance (B) (mmol)</td>
<td>1.6</td>
<td>(-0.9)-4.1</td>
<td>-3.4</td>
<td>-4.5-(-2.2)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

a, true intestinal calcium absorption; a, as fraction of dietary calcium; b, net intestinal calcium absorption; β, as fraction of dietary calcium; T\(_\text{Ca}\)/GFR, maximal active tubular calcium reabsorption in relation to GFR.

NS, not significant.

**Calcium and bone homeostasis**

Dietary calcium intake was significantly higher in the patients compared with controls (P < 0.01). Calcium absorption was significantly increased, absolute true absorption (P < 0.0001) as well as net absorption (b: P < 0.0001, β: P < 0.05). Urinary calcium excretion was not significantly different from control values. Fractional reabsorption of filtered calcium was unchanged, but T\(_\text{Ca}\)/GFR was significantly reduced in the patient group (P < 0.001). Calcium balance was positive in the patient group, significantly better than balance in the control group (P < 0.001) (Table 2). In bone, turnover was dramatically reduced. Resorption rates as well as mineralization rates were significantly reduced to very low values (P < 0.001 and P < 0.05 respectively), resorption rates relatively more so than formation rates (Table 2). In contrast, levels of bone resorption markers were in the upper normal range, not different from normal values (Table 3). The marker of collagen formation serum PICP, however, was significantly reduced (P < 0.05), as was another marker of bone formation, serum osteocalcin (P < 0.05). The ‘traditional’ marker of bone formation, serum AP, was not significantly different from normal values (Table 3). Data on serum ICTP and serum PICP were missing for 1 patient, and another was left out of the analysis of ICTP results because of an extreme value (36.9 contra mean of 3.26).

**Phosphate homeostasis**

Dietary phosphate was significantly higher in the patients (P < 0.01). Intestinal phosphate absorption and urinary phosphate excretion were not different from control values. In addition, renal filtration and reabsorption of phosphate as well as T\(_\text{Ca}\)/P/GFR were not different from normals. Phosphate balance was normal (Table 4).

**Table 3** Values (mean and 95% c.i.) of biochemical markers of bone resorption and bone formation in 15 vitamin D-treated hypoparathyroid patients compared with values from 12 age- and sex-matched normal controls. (24-h urinary OHP excretion was compared with standard laboratory normal range.)

<table>
<thead>
<tr>
<th>Hypoparathyroid</th>
<th>Mean</th>
<th>95% c.i.</th>
<th>Normal</th>
<th>Mean</th>
<th>95% c.i.</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-ICTP (mg/l)</td>
<td>3.3</td>
<td>2.7-3.9</td>
<td>3.4</td>
<td>2.7-4.1</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>24-h-U-OHP (mmol)</td>
<td>146</td>
<td>120-172</td>
<td>125</td>
<td>60-190</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>S-PICP (mg/l)</td>
<td>93.7</td>
<td>79.2-108.2</td>
<td>113.8</td>
<td>97.4-130.2</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>S-Osteocalcin (mg/l)</td>
<td>8.5</td>
<td>6.5-10.4</td>
<td>13.8</td>
<td>8.4-19.2</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>S-AP (U/l)</td>
<td>165</td>
<td>126-205</td>
<td>141</td>
<td>117-164</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

S-, serum value; U-, urine value. NS, not significant.
Magnesium homeostasis

Dietary intake of magnesium was significantly higher in the hypoparathyroid patients than in the control group (P < 0.001). In spite of this, the intestinal magnesium absorption was not significantly changed from control values. Urinary magnesium excretion was not significantly different in the two groups. The active renal magnesium reabsorption, however, was significantly reduced compared with normal reference values (P < 0.001), but fractional renal reabsorption of filtered magnesium was normal. The magnesium balance was more negative than balance in the control group, though not significantly different from controls (Table 5).

Discussion

The vitamin D-treated hypoparathyroid patients were properly vitamin D substituted with high serum 1,25-dihydroxyvitamin D and normal serum levels of calcium, which normally is the aim of the treatment. All patients had been treated for a long period, i.e. all should be in a steady state regarding mineral and bone metabolism.

Calcium and bone homeostasis

All patients were normocalcaemic, meaning that none of the observed effects were caused by abnormal serum levels of calcium. The high daily calcium intake in the patient group was due to the calcium supplementation. The high calcium intake as such might increase absolute intestinal absorption, but the fractional intestinal absorption should decrease. In this study both true and net calcium absorption were increased compared with normals, supporting the stimulatory role of the high serum levels of 1,25-dihydroxyvitamin D on intestinal calcium absorption (9, 24, 25). Hefti et al. (26) examined the effect of vitamin D treatment on hypoparathyroid rats and found that the intestinal absorption was unchanged and that the improvement in serum calcium levels caused by vitamin D treatment was due to increased bone resorption, as the increase was in the fasting calcium levels. On the other hand,

Table 4 Results (mean and 95% c.l.) from phosphate balance studies in 8 vitamin D-treated hypoparathyroid patients, compared with values obtained from a control group of 12 normal age- and sex-matched persons.

<table>
<thead>
<tr>
<th></th>
<th>Hypoparathyroid</th>
<th>Normal</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>95% c.l.</td>
<td>Mean</td>
</tr>
<tr>
<td>Diet (D) (mmol/day)</td>
<td>55.7</td>
<td>39.7–71.7</td>
<td>32.6</td>
</tr>
<tr>
<td>Faeces (F) (mmol/day)</td>
<td>35.3</td>
<td>24.5–46.2</td>
<td>16.5</td>
</tr>
<tr>
<td>Intestinal absorp. (b) (mmol/day)</td>
<td>20.4</td>
<td>6.7–34.0</td>
<td>16.1</td>
</tr>
<tr>
<td>Intestinal absorp. (β) (fractional)</td>
<td>0.34</td>
<td>0.12–0.56</td>
<td>0.48</td>
</tr>
<tr>
<td>24-h-urine phosphate (U) (mmol/day)</td>
<td>24.3</td>
<td>16.6–32.0</td>
<td>19.9</td>
</tr>
<tr>
<td>Renal filtered phosphate (mmol/day)</td>
<td>145.5</td>
<td>123.9–167.3</td>
<td>148.2</td>
</tr>
<tr>
<td>Renal reabs. phosphate (mmol/day)</td>
<td>127.5</td>
<td>99.3–155.6</td>
<td>128.3</td>
</tr>
<tr>
<td>TmP/GFR (mmol/l)</td>
<td>1.03</td>
<td>0.91–1.15</td>
<td>1.07</td>
</tr>
<tr>
<td>Phosphate balance (β) (mmol/day)</td>
<td>−3.93</td>
<td>(−12.9)–5.1</td>
<td>−3.8</td>
</tr>
</tbody>
</table>

b, net intestinal calcium absorption; β, b as fraction of dietary calcium; TmP/GFR, maximal active tubular phosphate reabsorption in relation to GFR.

NS, not significant.

Table 5 Results (mean and 95% c.l.) from magnesium balance studies in 8 vitamin D-treated hypoparathyroid patients, compared with values obtained from a control group of 12 normal age- and sex-matched persons.

<table>
<thead>
<tr>
<th></th>
<th>Hypoparathyroid</th>
<th>Normal</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>95% c.l.</td>
<td>Mean</td>
</tr>
<tr>
<td>Diet (D) (mmol/day)</td>
<td>15.2</td>
<td>11.4–18.9</td>
<td>9.3</td>
</tr>
<tr>
<td>Faeces (F) (mmol/day)</td>
<td>12.8</td>
<td>9.0–16.5</td>
<td>6.3</td>
</tr>
<tr>
<td>Intestinal absorp. (b) (mmol/day)</td>
<td>2.4</td>
<td>(−1.2)–6.0</td>
<td>3.1</td>
</tr>
<tr>
<td>Intestinal absorp. (β) (fractional)</td>
<td>0.13</td>
<td>(−0.15)–0.40</td>
<td>0.32</td>
</tr>
<tr>
<td>24-h-urine magnesium (M) (mmol/day)</td>
<td>4.6</td>
<td>3.6–5.6</td>
<td>4.4</td>
</tr>
<tr>
<td>Renal filtered magnesium (Mm) (mmol/day)</td>
<td>61.1</td>
<td>56.1–66.2</td>
<td>71.0</td>
</tr>
<tr>
<td>Renal reabsop. magnesium (Mm) (mmol/day)</td>
<td>57.8</td>
<td>51.9–63.7</td>
<td>66.5</td>
</tr>
<tr>
<td>TmMg/GFR (mmol/l)</td>
<td>0.46</td>
<td>0.42–0.49</td>
<td>0.55</td>
</tr>
<tr>
<td>Magnesium balance (β) (mmol/day)</td>
<td>−3.4</td>
<td>(−8.1)–1.4</td>
<td>−1.3</td>
</tr>
</tbody>
</table>

b, net intestinal calcium absorption; β, b as fraction of dietary calcium; TmMg/GFR, tubular magnesium reabsorption in relation to GFR.

NS, not significant.
Neer et al. (27) found that the increase was due to increased intestinal absorption in a study of five hypoparathyroid humans.

In spite of the increased intestinal calcium absorption, urinary calcium excretion was not increased compared to control values. Renal tubular calcium reabsorption is regulated in the proximal part of the nephron by two different mechanisms (17, 19, 23, 28, 29): a passive reabsorption in the proximal part of the proximal tubules with no maximum and independent of PTH (23, 30), and an active distal rate-limited mechanism with a maximum value \( T_{\text{mCa}}/\text{GFR} \) stimulated by PTH, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D (18, 23, 31, 32). According to this, we expected \( T_{\text{mCa}}/\text{GFR} \) to increase following the high levels of 1,25-dihydroxyvitamin D. However, we found that \( T_{\text{mCa}}/\text{GFR} \), the estimate of active renal calcium reabsorption, was significantly lower than normal. The total fractional reabsorption of filtered calcium was unchanged from the normal reference value, and since filtered amount of calcium was unchanged (normal serum calcium) this could imply two things: that vitamin D metabolites have no effect on active renal calcium reabsorption in the absence of parathyroid hormone, and/or that PTH is the most important hormone in the regulation of active calcium reabsorption. Furthermore, as filtered, net reabsorbed and excreted amounts of calcium were normal and active reabsorption was lower than control values, the other part of the calcium reabsorption, the proximal PTH-independent calcium reabsorption (23, 30, 33), must be increased. This could imply that the proximal reabsorption was influenced by the high vitamin D levels, supporting the findings of Puschett et al. (34, 35) who found such a relationship in dog experiments.

Vitamin D receptors have been found in osteoblasts, and in normal subjects vitamin D stimulates both number and activity of osteoblasts (5, 6, 36, 37). PTH receptors have also been found on osteoblasts, but not on osteoclasts (2, 6). PTH normally exposes the bone surface and prepares it for osteoclastic reabsorption by an effect on lining cells (38), and increases activation frequency. PTH stimulates osteocytic bone resorption and is important in the transportation of calcium from bone cells into the extracellular fluid, the exchangeable calcium pool (38), with a stimulating effect of vitamin D on calcium outflow (38). Malleruche et al. (39) found that vitamin D stimulated the activity of osteoblasts and that PTH stimulated the number of osteoblasts. Earlier calcium kinetic studies in patients with untreated HP have shown low bone turnover and low urinary OHP excretion (1, 40, 41). In our group of long-term vitamin D-treated hypoparathyroid patients we found that bone mineralization rate as well as bone resorption rate—and thereby bone turnover—were (still) significantly decreased after the vitamin D treatment. Bone mineralization rate was higher than bone resorption rate resulting in a more positive bone balance in the patient group compared with the normal group. However, the high levels of vitamin D as such were not able to accomplish the expected stimulating effect on matrix formation and mineralization that has been found in subjects with normal PTH levels (42, 43). Previous studies (44–46) have demonstrated low levels of biochemical markers of bone formation in untreated hypoparathyroid patients. Yoneda et al. (46) found that short-term vitamin D treatment in hypoparathyroid patients increased serum osteocalcin, but the levels were still lower than in normal subjects. In the present study on the long-term effect of vitamin D treatment, we also found that serum osteocalcin was significantly lower compared with normals—in spite of vitamin D treatment. This means that the stimulating effect of vitamin D on osteocalcin secretion in normals (47) was not found when PTH is missing. Serum osteocalcin is presumed to reflect total osteoblast activity, meaning that the lack of increase in serum osteocalcin levels despite vitamin D treatment could be due to either an interdependency between vitamin D and PTH or the result of a lack of PTH-induced increase in osteoblast numbers in spite of a stimulating effect of vitamin D on osteoblastic activity. Serum levels of the marker of collagen formation, serum PICP, were also significantly reduced. Gram et al. (47) found an increase in serum PICP levels as well as in serum osteocalcin levels when treating normal subjects with calcitriol for a week. Again it seems as if the stimulating effect of vitamin D is missing when PTH is absent. Duda et al. (45) found low levels of serum AP in untreated HP. We found that vitamin D-treated hypoparathyroid patients had normal levels of serum AP compared with controls. In histomorphometric analysis of bone biopsies from these vitamin D-treated hypoparathyroid patients (48) we also found bone turnover at tissue level reduced, but not to the same extent. The discrepancies in biochemical markers of bone turnover as well as in mineralization rate estimated by calcium kinetics and histomorphometry could be explained by the following arguments: histomorphometric analysis estimates bone formation rate (adjusted appositional rate) correcting for amount of osteoid-covered surface. This means that if vitamin D treatment independently results in acceleration of the mineralization of bone matrix, corresponding to normalization in serum levels of AP, this gives a relatively high estimate of bone formation rate. On the other hand, the calcium kinetic estimate of bone formation rate at organ level reflects calcium fluxes in bone and thereby the activity in total bone tissue—which is low—corresponding to the low values of serum PICP and serum osteocalcin as well as to the histomorphometric data on formative activity. This implies that vitamin D is the main regulator of AP secretion but not directly correlated to formative activity in bone. Serum osteocalcin and serum PICP on the other hand, are markers of mechanisms regulated by both vitamin D and PTH, correlating well with the total bone formative activity.
This suggests that matrix formation is regulated by vitamin D and PTH whereas the mineralization of newly formed bone matrix is controlled by vitamin D. The coupling phenomenon would explain why mineralization was low in spite of the high vitamin D and the normal serum AP levels. It was surprising that values of bone resorption markers were not reduced in the patients: 24-h urinary OHP excretion as well as serum ICTP were in the upper half of the normal range, contrasting with the calcium kinetic results of low resorption rate, and also results from earlier studies of low urinary OHP excretion in untreated hypoparathyroid patients (2). An explanation could be that the high levels might be due to an over-all stimulation of collagen metabolism in these patients, but serum ICTP has been found to be specific for metabolism of bone collagen, and levels of serum PICP, a marker of collagen formation, were low. The relatively high levels might be the result of decreased degradation rate, but this is not the case as all patients had normal liver and kidney function. Earlier studies on the effects of short-term vitamin D treatment in normal humans have shown conflicting results (36, 49). A possible explanation could be that these markers of bone resorption do not reflect every phase of bone resorption in hypoparathyroid patients. The resorption markers could be markers of specific phases in the bone resorption process, probably the early osteoclastic phase. This theory is supported by the data on bone metabolism at tissue level (histomorphometric analysis of bone biopsies) in these patients (48), where results from the early phases in bone resorption were almost normal and the defects in bone resorption emerged in the later phases of bone resorption.

**Phosphate homeostasis**

Various balance studies (19, 49) have found that vitamin D and hyperparathyroidism increase intestinal phosphate absorption while HP as well as vitamin D deficiency is followed by low intestinal phosphate absorption. We found that intestinal phosphate absorption was normal, which might be the net result of opposing effects of low PTH levels and high 1,25-dihydroxyvitamin D levels. Serum levels of phosphate were normal in the patient group and were not, therefore, causing any of the observed changes. Earlier studies of untreated hypoparathyroid patients found high levels of serum phosphate, low urinary phosphate excretion and high values of $T_n\text{P/GFR}$ (1), due to the missing phosphaturic effect of PTH (20, 33, 50). The normal serum phosphate levels, therefore, must be an effect of vitamin D treatment. In the kidney, phosphate is reabsorbed mainly from the proximal tubules by an active rate-limited process with a maximum value ($T_n\text{P/GFR}$), but a small part is reabsorbed from the distal tubules (29, 32, 33, 51). The active reabsorption is stimulated by both 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D (28, 34, 35, 52) and inhibited by PTH (33, 50). Theoretically, high levels of vitamin D as well as the lack of PTH should result in increased values of $T_n\text{P/GFR}$. However, several authors have postulated an interdependence between the two hormones in renal phosphate handling. The effect of the two hormones is, however, still not fully clarified (28, 31, 33, 53). Our findings of normal $T_n\text{P/GFR}$, urinary phosphate excretion and serum phosphate suggest that the stimulating effect of vitamin D on renal phosphate reabsorption was missing in the absence of PTH. These results support the findings of an experiment by Popovtzzer et al. (53) on rats. In all, the earlier presented abnormal phosphate homeostasis in untreated hypoparathyroid patients was apparently normalised by the vitamin D treatment, resulting in a normal phosphate balance.

**Magnesium homeostasis**

Serum levels of magnesium were normal. The serum value is not, however, a good parameter of body magnesium status as it decreases late in a state of deficiency. The small but significant increase in dietary magnesium in the patient group was due to the magnesium content of the tablets, where magnesium is an ingredient in the bulk material. Balance studies in patients with vitamin D poisoning demonstrated a high magnesium absorption (19). In a balance study of patients with intestinal by-pass operation examined before and after vitamin D treatment, Charles et al. (54) found that vitamin D treatment increased intestinal magnesium absorption. Several balance studies have shown (19) that hyperparathyroidism is followed by high intestinal magnesium absorption and that the absorption decreased after parathyroidectomy. Studies on magnesium absorption in hypoparathyroid patients are not available. In this study of vitamin D-treated hypoparathyroid patients we did not find a stimulating effect of vitamin D on intestinal magnesium absorption. This implies that high serum levels of 1,25-dihydroxyvitamin D were not able to increase intestinal magnesium absorption when parathyroid hormone was missing, implying a direct role for PTH in the intestinal magnesium absorption. This would be a new aspect of PTH effects, as the effect of PTH on the intestinal absorption of the three minerals has been thought by many to be indirect through its stimulating effect on serum levels of 1,25-dihydroxyvitamin D (24, 43, 55). The effect of PTH and vitamin D on renal handling of magnesium is not fully clarified. PTH has been shown to increase renal reabsorption of magnesium (22), but abnormal serum values have not been demonstrated in patients with hyperparathyroidism (19). Vitamin D has also been suggested to increase renal magnesium reabsorption (56, 57). The significantly reduced values of $T_{\text{Mg/GFR}}$ found in this study support a stimulating effect of PTH on renal magnesium reabsorption while the low $T_{\text{Mg/GFR}}$ in combination with normal urinary
magnesium excretion suggests the existence of an additional mechanism for magnesium reabsorption, stimulated by vitamin D.

In summary, we found that calcium balance in long-term vitamin D-treated hypoparathyroid patients was significantly more positive than calcium balance in normal subjects. Intestinal calcium absorption was increased but serum calcium was normal. This appears to be the result of the lowered renal calcium reabsorption and of a reduced flux of calcium from bone, probably due to lack of PTH. In bone, turnover was (still) low despite vitamin D treatment, which was reflected in low serum levels of biochemical markers of bone formation—except for the levels of AP which were normal(ized). Levels of bone resorption markers did not correspond to the low bone resorption rate, and the lack of PTH seems to induce a defect in the bone resorption process. This study shows that vitamin D treatment cannot normalize bone metabolism in hypoparathyroid patients and that PTH is necessary for a normal effect of vitamin D on bone remodelling. Phosphate balance was normal and the abnormalities found in earlier studies of phosphate homeostasis in untreated hypoparathyroid patients apparently were normalized by the vitamin D treatment. We found negative magnesium balance and decreased renal magnesium reabsorption implying a specific role of PTH in the magnesium homeostasis. The results suggest that PTH plays a direct role in intestinal absorption and renal reabsorption of the three minerals and that PTH may be necessary for 1,25-dihydroxyvitamin D to exert its effect on the intestinal and renal regulation of mineral homeostasis.

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